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Abbreviations: ABI: ankle-brachial index, IMT: intima media thickness.

Abstract

Background: Epidemiological evidence of the effects of long-term exposure to air pollution on the chronic processes of atherogenesis is limited.

Objective: We investigated the association of long-term exposure to traffic-related air-pollution with subclinical atherosclerosis, measured by carotid intima media thickness (IMT) and ankle-brachial index (ABI).

Methods: We performed a cross-sectional analysis using data collected during the re-examination (2007-2010) of 2,780 participants in the REGICOR study, a population-based prospective cohort in Girona (Spain). Long-term exposure across residences was calculated as the last 10 years' time-weighted average of residential NO_2 estimates (based on a local-scale land-use regression model), traffic intensity in the nearest street, and traffic intensity in a 100m buffer. Associations with IMT and ABI were estimated using linear regression and multinomial logistic regression, respectively, controlling for sex, age, smoking status, education, marital status and several other potential confounders or intermediates.

Results: Exposure contrasts between the 5th and 95th percentiles for NO_2 ($25\mu g/m^3$), traffic intensity in the nearest street (15,000 vehicles/day) and traffic load within 100m (7,200,000 vehicle-meters/day) were associated with differences of 0.56% (95% CI: -1.5, 2.6%), 2.32% (95% CI: 0.48, 4.17%) and 1.91% (95% CI: -0.24, 4.06) percent difference in IMT, respectively. Exposures were positively associated with ABI>1.3, but not ABI<0.9. Stronger associations were observed among those with high education and in men > 60 years of age.

Conclusions: Long-term traffic-related exposures were associated with subclinical markers of atherosclerosis. Prospective studies are needed to confirm associations and further examine differences among population subgroups.

Introduction

Air pollution from traffic and other sources is an established cause of premature mortality (Brook et al. 2010). A relevant part of this environmental burden of disease relates to cardiovascular diseases (CVD), which were responsible for 10% of total Disability Adjusted Life Years in 2004 and the leading causes of death worldwide in 2008 (World Health Organization 2008). A common feature of this group of diseases is atherosclerosis, a chronic and degenerative process that mainly occurs in large and medium-sized arteries and is characterized by asymmetric focal thickenings of the innermost layer of the artery, the intima. The development of atherosclerosis is the result of the total cumulative exposure to atherogenic risk factors such as hypertension, high cholesterol, diabetes, obesity, smoking, physical inactivity, and other lifestyle factors, and their interactions with genetic susceptibility (Lusis 2000). Acute events such as myocardial infarction or stroke can be triggered by short-term exposure to air pollution (Brook et al. 2010). However, whether and how ambient air pollution contributes to atherogenesis is subject to debate. While experimental studies on animals provide strong evidence for a causal atherogenic role of air pollution, particularly in obese mice (Sun et al. 2005), evidence from epidemiological studies is limited.

The long subclinical phase of atherosclerosis makes it possible to investigate the determinants of the vascular pathology long before its clinical manifestation. The association between air pollution and intima media thickness (IMT), an established marker of subclinical atherosclerosis, was reported for the first time in volunteers participating in two clinical trials in California (Künzli et al. 2005). Two population-based cross-sectional analyses, namely the Heinz Nixdorf

Recall study in Germany (Bauer et al. 2010) and the MESA Cohort in the United States (Diez Roux et al. 2008), also reported associations between air pollution and IMT, while a study of young adults in the Netherlands found no association (Lenters et al. 2010). So far, only one longitudinal study has been published, based on heterogeneous samples of volunteers participating in five clinical trials (Künzli et al. 2010), including the two trials of the first cross-sectional study published on this topic (Künzli et al. 2005). Findings from the study suggested a possible role of ambient air pollutants, indicated by $PM_{2.5}$ and living close to busy highways, in the progression of IMT (Künzli et al. 2010).

Most previous studies have reported that associations between air pollution and IMT differed among population subgroups (Künzli et al. 2011). However, subgroup patterns have not been consistent, thus, a clear understanding of susceptibility factors is still lacking. Human studies have not investigated whether diet modifies effects of air pollution – a plausible hypothesis given evidence from animal studies (Sun et al. 2005) and effects of diet on oxidative stress and systemic inflammation that are likely to contribute to the systemic effects of ambient air pollution (Brook et al. 2010).

The Mediterranean region of Girona, and Spain in general, has one of the lowest cardiovascular mortality rates in Europe, despite very high prevalence of conventional cardiovascular risk factors (Masia et al. 1998), a paradox that may be explained in part by protective effects of the Mediterranean diet (Martínez-González et al. 2011). Our study aimed to investigate the association between long-term exposure to traffic-related air pollution and subclinical atherosclerosis in Spain, and modification of this association by diet and other factors identified

in previous studies (Künzli et al. 2011). We investigated this in the follow-up of participants of three population-based cohorts of the REGICOR study (Grau et al. 2007). Subclinical atherosclerosis was measured by carotid intima media thickness (IMT), a validated marker of atherosclerosis (Coll and Feinstein 2008). Additionally we measured ankle brachial index (ABI), a marker of the presence and severity of peripheral artery disease. Both IMT and ABI are associated with cardiovascular events and mortality (Ankle Brachial Index Collaboration 2008; Lorenz et al. 2007). In the absence of particulate matter measurements in this region, we used estimates of 10-year average home outdoor nitrogen dioxide (NO_2) concentrations and residential traffic intensity as markers of exposure to local traffic-related air pollutants.

Methods

REGICOR-Air is a cross-sectional study nested in the REGICOR cohort study (Grau et al. 2007). We used data from the follow-up of three population-based cohorts originally enrolled in 1995, 2000 and 2005 (response rate 72.4, 70.0 and 73.8%, respectively) from 12 towns that represent the geographic diversity of the Girona Province and have large contrasts in ambient air pollution levels (see map in Supplemental Material Figure S1). During 2007-2010, the participants residing in these towns, who were alive and not institutionalized, were invited to participate in REGICOR-Air, and the response rate was approximately 82%. In addition to extensive health status re-assessment, IMT and ABI were measured for the first time. Address histories for the past 10 years were collected by questionnaires and each address was geocoded at the front door level. This study was approved by the Hospital del Mar Research Institute ethics committee and participants gave written informed consent.

IMT was measured by three trained and certified sonographers using ultrasound examinations of the left and right carotid arteries. Standardized scan and image analyses protocols were used. The scan protocol entailed the right and left carotid arteries. In each artery, predefined longitudinal segments of the common carotid 1 cm proximal to the dilation, the carotid bulb and the internal carotid 1cm. distal of the flow divider were imaged. For imaging, an Acuson Aspen ultrasound instrument (Acuson-Siemens, Erlangen, Germany) equipped with an L7 5-12MHz transducer and dedicated REGICOR application scan protocol (AMC IMAGELAB, University of Amsterdam/Technical University Eindhoven, The Netherlands) were used. A still image of each arterial segment was saved as a DICOM (Digital Information and Communication in Medicine) file. These source files were locally stored and securely transferred to IMAGELAB, where trained and certified sonographers analyzed the images using validated "eTrack REGICOR" software (Department of Physiology and Vascular Medicine Academic Medical Centre, Amsterdam) (de Groot et al. 2008). IMT was defined as the average distance between the lumen-intima and media-adventitia interfaces in a given 1cm segment of the artery far wall. The main outcomes were: a) the mean of the IMT for the left and right common carotid arteries (IMTcca) and b) the mean of the IMT of for the left and right common carotid arteries, internal carotid arteries, and carotid bulbs (IMT6seg). These two outcomes were assessed separately because of differences in segments' cellular constituents –with more foam cells in the common carotid artery and more cholesterol-rich plaques in the carotid bulb and the internal carotid artery (Gállego Pérez-Larraya et al. 2012)– and in risk factors for IMT (Polak et al. 2010), which suggest that etiology may differ according to location. Between-sonographer and between–visit variability were evaluated based on repeated IMT measurements conducted at two visits, two weeks apart, by up to three sonographers, in 42 participants.

Systolic blood pressure was measured in a supine position after a 5-minute rest in the brachial artery of both arms and the posterior tibial and dorsalis pedis arteries of both legs, using a continuous Doppler device. Right and left ABI were calculated as the ratio of the highest leg pressure to the highest brachial pressure in the corresponding arm, and the lowest of the two ABI ratios were categorized as low (<0.9), normal (0.9-1.3), or high (>1.3) for analysis (Ankle Brachial Index Collaboration 2008; McDermott et al. 2005).

Adherence to Mediterranean diet was measured by a 10-point index based on sex-specific intake tertiles of 8 beneficial (cereals, fruits, vegetables, legumes, seafood, nuts, moderate red wine) and 2 detrimental (meats, dairy products) food groups, and categorized in quartiles. Modifications such as excluding low-fat dairy products or white meats from the detrimental foods, or incorporating additional unhealthy food groups (e.g. soft drinks, salty snacks, pastries) as detrimental components had no meaningful impact on findings in sensitivity analyses (data not shown). A detailed description of the methodology used to measure adherence to Mediterranean diet in the REGICOR study is given by Schröder et al. (2004). The plausibility of reported dietary intakes was assessed based on disparities between reported energy intakes and estimated energy requirements (Mendez et al. 2011).

Participants with a clinical history of CVD (myocardial infarction, stroke, angina, catheterization, angioplasty, bypass surgery, or amputation due to circulatory problems) were excluded (n=227) since medication use or altered health behaviors among these subjects may have influenced IMT/ABI measures obtained for this study.

Exposure Assessment

We estimated the 10-year time-weighted average of the home outdoor concentrations of NO_2 for each participant using land use regression models. In the absence of air quality data in most of these towns, we conducted an extensive monitoring campaign using NO_2 passive samplers to validate model estimates. We measured NO_2 in the balcony of 562 participants' homes for one month in the spring and fall between June 2007 and July 2009. Homes were selected to cover a broad range of traffic-related pollution and urban settings (e.g. low and high building-density areas), to be representative of the residential locations of the cohort participants and to be well distributed across the towns. We adjusted for temporal variability using monthly mean NO_2 concentrations collected at a fixed location in each town for at least one year. NO_2 annual means were derived multiplying the monthly means at each location by the ratio of the annual mean to the same month mean NO_2 at the town's fixed location.

To predict NO_2 at each participant's residence we used land use regression (LUR) models based on NO_2 annual means and data on traffic intensity, bus routes and stops, distance to traffic, land cover, building density, and other GIS-derived variables. Given the geographic diversity of the study area and differences in the availability of GIS data among towns, we divided the study area into seven subareas, i.e. groups of adjacent towns. LUR models were derived for each group by supervised forward linear regression following the methodology described by Rivera et al. (2012). The models explained between 33 and 63% of measured NO_2 (cross-validation R^2 0.32 and 0.61, respectively) (Supplemental Material, Table S1 and Figure S2).

We estimated the outdoor annual mean NO_2 at each residential location by applying the LUR models to the address geocode. NO_2 concentrations were back-extrapolated to the time period of residence in each address using daily means at a fixed urban background monitoring location. Finally, for each participant we calculated the time-weighted average of NO_2 estimates across all residences in the 10 years prior to the IMT measurement (10yr NO_2 exposure). Time periods when participants lived at addresses that were geocoded with low precision or were outside of the study area were excluded when deriving the 10yr NO_2 exposure. Participants who lived in the study towns less than 6 years or had high precision geocodes (at the front door level) for fewer than 6 years (n=365) were excluded from the main analyses.

We also used traffic proximity markers as surrogates of air pollution exposure in independent analyses. Traffic intensity was available from local registries and the 2007 Urban Mobility Plan for Girona(2007) and was assigned to the central road network used within ESCAPE (Hoek et al. 2012). Traffic counts were conducted at approximately 670 streets with missing traffic information to complete the traffic inventory. The traffic intensity assessment is described in detail elsewhere (Rivera et al. 2012). For each address we calculated the traffic intensity at the nearest street and the traffic load (sum of traffic intensity multiplied by length of road segment in all segments) in a 100m buffer and derived 10-year average values for each participant.

All GIS calculations were done using ArcGIS 9.2 (ESRI, Redlands, CA).

Statistical Analysis

Crude and adjusted associations of IMT (natural log-transformed to reduce heteroscedasticity) with individually assigned air pollution exposures were estimated using linear regression models. Linear models were compared with additive models with smoothing splines using the generalized additive model (GAM) function in the *mgcv* R package and linearity was confirmed. We analyzed ABI using multinomial logistic regression (Hosmer and Lemeshow 2004). We initially adjusted our models by age and sex. Age was a main determinant of both outcome and exposure. Thus, in a second step, we analyzed the association of the potential confounders with both outcome and exposure variables adjusting by age and sex. Variables associated with both, namely, smoking status, education level (as a proxy of socioeconomic status) and marital status were included in a minimal adjustment set. The association between exposure and IMT was strongly confounded by age. To determine whether age was an effect modifier the interaction term age*exposure was introduced in the model and it was not significant (p>0.05). We also evaluated whether the effect of age differed for men and women. This was the case so we added the sex-age interaction to our minimal adjustment set (Model 1). We further adjusted by other potential confounders [physical activity (weekly energy expenditure in the leisure-time based on the Minnesota questionnaire (Elosua et al. 2000)expressed in tertiles of metabolic equivalents), diet (adherence to Mediterranean diet and plausibility of reported diet), SES at the census tract level (percentage of residents with < secondary school education in the tract where participants resided the longest, based on the 2001 census), and occupational status] and results (not shown) did not differ from those of the Model 1. Finally, in addition to the variables listed above, we also included potential intermediates [BMI, HDL, waist circumference, systolic and diastolic blood pressure, and medication treatment (lipid-lowering, anti-hypertensives, antiplatelets and anticoagulants based on self-report and medical records)] to explore the effects of air pollution

that do not follow these pathways (Model 2). Adjustment variables were entered in the models either as continuous variables or dummy variables for all categories according to Table 1 and Supplemental Material, Table S2.

We did not adjust the exposure-outcome associations for area of residence in the main analyses since the study area was relatively small (65x70Km), data collection in every town .was done by the same team using the exact same procedures, and it would partially remove the exposure contrast corresponding to between-town variability. However, we explored the sensitivity of the results to the inclusion of area of residence (corresponding to the address of longest residence) as a random effect variable. The trend of the associations remained the same but the estimates were closer to the null and less precise (data not shown). This is consistent with a decrease in the exposure contrast, which makes the detection of an effect more difficult. Residual confounding by area is less likely b the estimates were adjusted by a large array of confounders, including area level confounders such as education at the census tract.

Because living near busy roads is also associated with traffic-related noise, we adjusted for road traffic noise in a subsample of participants with available noise data (n=1084) (Foraster et al. 2011). However, results were not sensitive to adjustment for noise (data not shown).

Results are expressed as the cross-sectional percent difference in IMT, and the relative risk ratio (RRR) of high and low ABI, associated with a 10-year exposure contrast corresponding to the difference between the 5th and 95th and percentiles in the study population. The RRR of high ABI is the prevalence of high ABI relative to normal ABI in exposed divided by the prevalence of

high ABI relative to normal ABI in non-exposed.

We tested effect modification by diet and factors identified as potential effect modifiers in previous studies, namely, age, sex, education level, smoking, diabetes, menopause, and medication treatment, using stratified analysis. We report the effect estimates of percent differences in IMT by strata of the potential effect modifiers.

To assess the sensitivity of the results to the NO_2 LUR models, we used the annual mean NO_2 at the closest monitor within 100m and 200m of the residence, instead of the 10-year modeled NO_2, in two separate sensitivity analyses. Median distance to the closest NO_2 monitoring site was 90m. The sample size was restricted in these analyses because only those whose address of longest residence was within 100m and within 200m of a monitor were kept (e.g. 2,265 and 1,778 people lived within 200m of a monitor with data available on IMTcca and IMT6seg, respectively).

Analyses were done using Stata 10.1 (StataCorp, College Station, TX) and R 2.12 (http://www.R-project.org). The alpha level for statistical significance was set at 0.05.

Results

Information on IMTcca, IMT6seg and ABI was available for 2780, 2188 and 2738 participants, respectively. Fewer participants had IMT6seg measurements because of difficulties in analyzing

the images to obtain measurements from the internal carotid artery and carotid bulb (Gállego Pérez-Larraya et al. 2012). The characteristics of the study populations are summarized in Table 1. Participants were 32 to 86 years old. Percentages with low, medium and high education are consistent with Spain as a whole (census 2001). Participants included in the analyses did not differ from those excluded (participants who lived in the study towns less than 6 years or with clinical history of CVD) in terms of exposure levels (data not shown). The median IMTcca was 0.68mm (range 0.40–2.05mm). The repeatability study showed intraclass correlation coefficients for sonographers and visits of 0.83 for the IMTcca and of 0.77 for the IMT6seg. ABI was on average 1.10 (range 0.5–1.75) with 2.0% of the study population classified as having low ABI (<0.9) and 4.2% with high ABI (>1.3) (Table 1). Participants with low ABI were on average nine years older than participants with normal ABI, whereas those with high ABI did not differ in age from participants with normal ABI (data not shown). Inter- and intra-operator variability of ABI measurements were low, with intraclass correlation coefficients of 0.92 and 0.94, respectively.

The 10-year average home outdoor nitrogen dioxide concentrations varied from 5 to 48 $\mu g/m^3$ (Supplemental Material, Table S3) and its correlation with NO_2 at the address of longest residence was >0.99 over all subjects (Supplemental Material, Table S4), and 0.96 among those who moved at least once during the 10 years (data not shown). Ranges of 10-year time-weighted average values for traffic in the nearest street and traffic within 100m indicated substantial variability among participants overall (Supplemental Material, Table S3) and according to town of residence (Supplemental Material, Table S5). Traffic intensity showed higher spatial variability than NO_2 [(p75-p25)/p50 was 2.51 for 10-yr traffic in the nearest street, 1.56 for 10-yr traffic load in 100m, and 0.73 for 10-yr NO_2]. NO_2 and traffic exposure variables were

moderately correlated (correlation coefficients 0.52–0.72), and the two traffic variables had a correlation of 0.58 (Supplemental Material, Table S4).

In unadjusted models, nitrogen dioxide, traffic in the nearest street and traffic in the 100m buffer were strongly associated with the intima media thickness (both IMTcca and IMT6seg) (Table 2). Associations decreased after adjusting for age, and in general, further adjustment (Model 1 and Model 2) provided similar estimates. Associations between a $25\mu g/m^3$ increase in NO_2 (corresponding to the difference between the 5th and 95th percentiles of exposure) and both IMTcca and IMT6seg were positive but small and non-significant after adjustment. Models fully adjusted –including potential intermediates (Model 2)- indicated that a corresponding exposure contrast for traffic load within 100m (7,200,000 vehicle-meters/day) was associated with a 1.91% difference in IMTcca (95% CI: -0.24, 4.06) and a 2.06% difference in IMT6seg (95% CI: -0.09, 4.21). An increase of 15,000 vehicles/day on the nearest street was associated with a 2.32% difference in IMTcca (95% CI: 0.48, 4.17) and a 1.80% difference in IMT6seg (95% CI: 0.01, 3.59).

10-year NO_2 and both residential traffic indicators were associated with higher prevalence of high ABI (Table 3). Adjusted RRRs for high vs. normal ABI were 1.98 (95% CI: 1.09, 3.60) for a $25\mu g/m^3$ increase in NO_2; 1.89 (95% CI: 1.07, 3.34) for a 7,200,000 vehicle-meters/day increase in traffic load within 100m; and 1.70 (95% CI: 1.13, 2.57) for an increase of 15,000 vehicles/day on the nearest street. The RRR of low ABI compared to normal ABI were consistent with the null hypothesis for all exposures.

Effect modification

The association of all exposure markers with IMT differed across education level (Figure 1). In people with higher education, the association of 10-year exposure to air pollution with IMT was stronger (Model 2 with NO_2 and IMTcca). Increases of $25\mu g/m^3$ in NO_2, 7,200,000 vehicle-meters/day in traffic load in 100m, 15,000 vehicles/day on the nearest street, were associated with 4.6% (95% CI: 0.4, 8.9), 4.8% (95% CI: 0.7, 8.9) and 3.3% (95% CI: -0.02, 6.7) differences in IMTcca, respectively, among people with high education; -1.5% (95% CI: -5.1, 2.1), 1.3% (95% CI: -2.4, 5.0), 1.3% (95% CI: -1.8, 4.4) among people with secondary school; and 0.6% (95% CI: -2.8, 2.9), 0.5% (95% CI: -2.7, 3.8), 2.4% (95% CI: -0.6, 5.6) among people with primary school. Similar patterns were observed for the associations of exposure to air pollutants with IMT6seg. Effects estimates were also stronger in men older than 60 years compared to younger men, showing significant associations of NO_2 and traffic load in 100m with IMT (percent difference in IMTcca for NO_2, traffic load and traffic intensity were 4.3% (95% CI: 0.2, 8.4), 5.9% (95% CI: 1.6, 10.3) and 3.4% (95% CI: -0.07, 7.0) respectively, among men ≥60 and -1.5% (95% CI: -5.7, 2.7), -1.5% (95% CI: -5.7, 2.6) and 1.2% (95% CI: -2.7, 5.0) respectively, among men <60). No evidence of effect modification by smoking, medication treatment (Figure 1), menopause, diabetes (results not shown) or Mediterranean diet (see Supplemental Material, Figure S3) was observed.

Discussion

Our multivariate models revealed positive associations between three markers of long-term exposure to traffic-related air pollution, namely traffic load within 100m of the residence, traffic

intensity in the nearest street and modeled concentration of home outdoor NO_2 and carotid subclinical atherosclerosis in a random healthy (no history or current signs of CVD) population sample of the Spanish Mediterranean region. However, associations with NO_2 were weak and reached statistical significance only in the subgroups of people with high education level (IMTcca and IMT6seg) and men above 60 years (IMTcca). Both indicators of residential traffic were associated with approximately 2% thicker carotid intima-media. High exposures to NO_2 and traffic were also associated with higher prevalence of high ABI [RRR for high vs. normal ABI between 1.70 (95% CI: 1.13, 2.57) for traffic intensity in nearest street and 1.98 (95% CI: 1.09, 3.60) for NO_2]. To put these results in context with common risk factors for atherosclerosis, in the same study population included in main analyses, a 10 year difference in age, adjusted by confounders in "Model 2", was associated with an 8% (95%CI: 7.3, 9.0) difference in IMTcca and a RRR of 10.3 (95%CI: 10.0, 10.5) for high vs. normal ABI. Interestingly, age strongly confounded the association of pollution with IMT. Removing age adjustment from the main multivariate models resulted in substantially larger estimates, very similar to the crude associations. Atherosclerosis is a life-time process, thus, age is a strong correlate of the cumulated life-time exposure to air pollution, as well as the main predictor of atherosclerosis. Thus, adjustment for age may in part reflect over adjustment on exposure.

Our results suggest that long-term exposure to traffic-emitted pollution may contribute to carotid artery atherosclerosis. Given widespread exposure to high traffic, the potential association of long-term pollution with atherosclerosis has important public health implications. Ninety five percent of our study population was exposed to traffic intensity levels in the nearest street <15,000 vehicles/day, which is very low compared with other European cities. For example, in

larger cities such as Barcelona, many people live within very short distances of streets with traffic intensities of 50,000–100,000 vehicles/day (Amato et al. 2009). Likewise, levels of NO_2 can exceed 100μg/m^3 near streets with high traffic intensity. Expressing our results in terms of the 5th-95th percentile exposure contrast (25 μg/m^3 NO_2, 15,000 vehicles/day on the nearest street, or 7,200,000 vehicle-meters/day within 100m) thus reflects ranges that are frequently observed in European cities.

Our findings are consistent with previous reports of association of medium- to long-term exposure with subclinical atherosclerosis (Bauer et al. 2010; Diez Roux et al. 2008; Hoffmann et al. 2007; Künzli et al. 2005) and systemic inflammatory markers (Hoffmann et al. 2009a), though direct comparisons are limited given our use of NO_2 instead of PM mass as a marker of pollution. However, our effect estimates are in the same order of magnitude as in previous studies.

In contrast, the association between pollution and ABI was less clear. Low ABI (<0.9) is more clearly associated with mortality and cardiovascular disease than high ABI, but only high ABI was associated with traffic-related exposure in our study. Only 56 participants (2%) were classified as having low ABI [compared with 116 (4.2%) with high ABI], resulting in low power to detect an association. In the MESA study, 20-year exposures to $PM_{2.5}$ and PM_{10} were not associated with ABI as a continuous outcome, while 1-year mean PM_{10} exposures were associated with higher ABI "indicating less subclinical disease among persons with greater exposure" (Diez Roux et al. 2008). On the other hand, a study in Germany reported that living 50m from a major road compared to living more than 200m away was associated with an OR of

1.77 (95%CI: 1.01, 2.1) for peripheral arterial disease (ABI<0.9 or history of treatment for peripheral artery disease), while no associations were found with annual residential $PM_{2.5}$ (Hoffmann et al. 2009b).

The predictive power of ABI higher than 1.3 for cardiovascular risk has been less studied. It has been associated with calcification of the arterial wall, higher levels of many CVD risk factors (McDermott et al. 2005), higher risk of all-cause mortality and foot ulcers, and has been weakly associated with heart failure and stroke (Allison et al. 2008). Thus, there is evidence of higher cardiovascular risk both at low and high levels of ABI. We found that traffic-related exposure was associated with high ABI. To our knowledge, the association of air pollution with high ABI has not been investigated before. ABI is considered to be a marker for medial sclerosis, which is a specific form of arterial disease distinct from atherosclerosis (Alzamora et al. 2012). Since ABI is a ratio of systolic blood pressures, high ABI could result from low brachial pressure, or high ankle pressure, or both (Allison et al. 2008). Thus, if air pollution affects the upper and lower vascular beds differently, for example, by affecting brachial pressure at an earlier stage than ankle pressure, it could be associated with both high and low ABI. This hypothesis is consistent with our finding that high (vs. normal) ABI was not associated with IMT (adjusted RRR of 7.7; 95% CI: 1.6, 36.8, p-value: 0.01 for 1mm increase in IMTcca), while low (vs. normal) ABI was associated with thicker artery walls (adjusted RRR of 1.4; 95% CI: 0.3, 6.3 for 1mm increase in IMTcca).

In contrast to our findings, 20-year exposures to $PM_{2.5}$ and PM_{10}, in the MESA study were weakly associated with carotid IMT (1 to 3% differences in IMT for 12.5 $\mu g/m^3$ and 21$\mu g/m^3$

increases in 20-year average $PM_{2.5}$ and PM_{10}, respectively) but not with ABI (Diez-Roux 2008).

In Los Angeles, a $10\mu g/m^3$ increase in $PM_{2.5}$ at the current address was associated with a 4.2%

(95% CI: -0.2, 8.9) increase in IMT (Künzli et al. 2005). In a longitudinal study that included the

population of Künzli et al. (2005), a $10\mu g/m^3$ increase in $PM_{2.5}$ and living within 100m to a

highway were associated with 2.5µm/yr (95% CI; -0.31, 5.38) and 5.5µm/yr (95% CI; 0.13,

10.8) increases in IMT, respectively (Künzli et al. 2010). In the Heinz Nixdorf Recall study, a

$4.2\text{-}\mu g/m^3$ increase in one year average $PM_{2.5}$ and a 1,939 m increase in distance from a major

road were associated with a 4.3% (95% CI: 1.9, 6.7%), and 1.2% (95% CI: -0.2, 2.6%)

difference in IMT, respectively (Bauer et al. 2010). Distance to traffic was used as a maker of

traffic-related exposure thus a negative association with IMT was expected. A $5.2\ \mu g/m^3$ increase

in one year average PM_{10} was significantly associated with IMT in a study of over 2,000 civil

servants in London (Tonne et al. 2012). Positive, though not significant, associations of IMT

with measures of traffic intensity at the current address, but no association with a $25\text{-}\mu g/m^3$

increase in NO_2, were observed in a population-based study of 745 young adults in Utrecht

(Lenters et al. 2010).

Although associations with traffic as a surrogate of exposure appear to be more consistent across

studies than associations with pollutants, more research is needed to clarify the role of specific

constituents of air pollution on atherogenesis. In the study of young adults in Utrecht,

associations of NO_2, black smoke, $PM_{2.5}$ and SO_2 with IMT, pulse wave velocity, and

augmentation index were investigated, but significant associations were found only for NO_2 with

pulse wave velocity and augmentation index and for SO_2 with augmentation index (Lenters et al.

2010). The evidence on the health effects of NO_2 varies across studies. It has been reported to be

more strongly associated with cardiovascular mortality than $PM_{2.5}$, black smoke, and SO_2 (Beelen et al. 2008) and with cardiopulmonary mortality than PM_{10}, total suspended particles, black smoke, and SO_2 (Gehring et al. 2006), but Pope et al. (2002) found no association between NO_2 and cardiopulmonary mortality. The different associations observed between NO_2 and cardiopulmonary and cardiovascular outcomes across studies might reflect the suitability of using NO_2 as a marker of traffic-related pollution. While the general suitability of NO_2 has been questioned before, the possibility of it being site dependent has not been studied. It has been reported that the correlation of NO_2 with traffic intensity vary across locations (Raaschou-Nielsen et al. 2000). The correlation of NO_2 with the components of the traffic emissions cocktail that promote (assuming the association was causal) or are truly associated with cardiovascular outcomes might also vary across locations.

Our exposure estimates (both NO_2 and traffic) were based on data collected at participants' residences, instead of data from air quality monitoring stations as far away as 10km or more (Diez Roux et al. 2008). In addition, our traffic markers were based not only on proximity and road type classification (Allen et al. 2009; Hoffmann et al. 2009a), but also on actual traffic intensity derived from a dense traffic-count network. However, R^2s for our NO_2 models indicate some exposure measurement error (Basagaña et al. 2012). To evaluate the potential influence of measurement error (both bias and misclassification) introduced by the exposure models, we compared associations with our model-based estimates of 10-year average NO_2 with associations based on annual mean NO_2 at the closest monitor. Results (not shown) remained unchanged, which suggests that measurement error introduced by the model was negligible or comparable to the error associated with measures taken at the closest monitor.

Stronger positive associations in people with high education level were consistent across IMT measurements and across all markers of pollution. Larger estimated effects of traffic-related exposure on IMT were observed in men older than 60 years in all models. We did not find evidence of effect modification by Mediterranean diet, established risk factors for CVDs, or subclinical disease (indicated by medication treatment). Other studies have found heterogeneous associations across subgroups of age, sex, BMI, smoking status, socioeconomic status, town of residence and other cardiovascular risks factors (Bauer et al. 2010; Künzli et al. 2005; Lenters et al. 2010). The detection of interactions in epidemiologic studies is often underpowered and testing many interactions can also lead to a multiple comparison problem. However, our subgroup analysis was designed a priori, and although our results may be subject to the aforementioned problems, the accumulated evidence over different studies will help to identify susceptible subgroups.

In Girona, exposure to traffic-related pollutants was higher for people with high education level. Higher NO_2 concentrations were also found at the most privileged census tracts (see Supplemental Material, Figure S4). This has been reported before for South European cities (Cesaroni et al. 2010) where wealthy people live in downtown areas that are more polluted, in sharp contrast to what has been observed in North American (Gunier et al. 2003) and northern Europe (Chaix et al. 2006) where the most deprived bear the highest air pollution concentrations.

The people with higher levels of education were also younger, have lower blood pressure, BMI, glucose levels, lower LDL, a higher percent have quitted smoking and a lower percent have

never smoked compared to people with low and medium education. Stronger associations between air pollution and IMT among those with high versus low education were not explained by age interactions. This may indicate higher susceptibility but, more likely, a better detectability among people with fewer competing risks for atherosclerosis (due to less confounding). It is difficult to evaluate if this is supported by previous studies given that results are very heterogeneous. No effect modification by education level has been observed before for the association of $PM_{2.5}$ with IMT, ABI or coronary artery calcification (Diez Roux et al. 2008), neither for the association of NO_2 or $PM_{2.5}$ with IMT and arterial stiffness (Lenters et al. 2010). Whereas increasing effects of roadway proximity on aortic artery calcification have been reported for increasing income (trend P <0.01) (Allen et al. 2009).

Associations of $PM_{2.5}$ with systemic inflammation markers have been reported to be stronger in men, and more specifically, in highly educated men in Germany (Hoffman 2009). Slightly stronger associations with annual PM_{10} were estimated among men compared to women in the study in civil servants in London (Tonne et al. 2012). Contrary to our results for men>60, stronger associations between $PM_{2.5}$ and IMT were estimated for women>60 in the study in Los Angeles (Künzli et al. 2005). The difference in augmentation index associated with increased levels of NO_2 and $PM_{2.5}$ was also higher for young women in Utrecht (Lenters et al. 2010).

Associations with NO_2 or traffic load or intensity at the current address (at the time of examination) were weaker and less precise (data not shown) than associations with 10-year weighted averages across addresses, indicating exposure misclassification. Similar results were observed in the London study in which exposure at current address gave less precise estimates

compared to exposure averaged during one year before examination (Tonne et al. 2012). This highlights the relevance of using long-term exposure when studying effects on the IMT. On the other hand, our results remained unchanged when using exposure at the address of longest residence compared to 10-year averaged exposure (data not shown). Thus, for settings similar to the Spanish Mediterranean region, in terms of patterns of spatial distribution of NO_2 and low residential mobility (i.e. 80% of people did not change address in the 10 years), the exposure at the address of longest residence may be a good proxy for long-term exposure.

Limitations of our study include the cross-sectional design and the possibility of unmeasured confounding, including confounding related to environmental tobacco smoke, and the lack of information on daily activity patterns to assess time spent at home. Strengths include the large population-based sample size and the availability of data on health and potential confounders, the 10-year address histories used to estimate long-term NO_2 and traffic exposure markers, and the dense NO_2 and traffic monitoring network data used to estimate exposures at each participant's residences.

In conclusion, we found evidence supporting an association between long-term exposure to traffic and subclinical carotid atherosclerosis and high ABI levels in our study population, with stronger associations in people with high education. Longitudinal studies are needed to confirm whether air pollution contributes to the chronic processes of atherogenesis.

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Table 1. Descriptive statistics of the study population included in main analyses *n=2780*.
N (%) unless otherwise indicated.

Characteristic	N (%) unless otherwise indicated
IMTcca (mm), median ± IQR	0.68 ± 0.19
IMT6seg (mm), median ± IQR	0.67 ± 0.18
Ankle Brachial Index (minimum of left and right)	
ABI<0.9	56 (2.0)
ABI>1.3	116 (4.2)
Age (yrs), median ± IQR	58 ± 18
Sex (women)	1491 (53.6)
Education level	
Primary school or illiterate	1476 (53.1)
Secondary school	758 (27.3)
Technician or higher education degree	526 (18.9)
Occupational status	
Employed	1447 (53.1)
Inactive or house keeper	358 (12.9)
Retired	852 (30.7)
Unemployed	68 (2.5)
Smoking status	
Never smoker	1202 (54,5)
Former smoker	628 (28,5)
Current smoker	377 (17,1)
Marital status	
Single	165 (5.9)
Married / living together	2178 (78.4)
Divorced	171 (6.2)
Widow	247 (8.9)
Other	9 (0.3)
Body mass index, median ± IQR	26.6 ± 5.5
Waist circumference (cm), median ± IQR	93 ± 17
High density lipoprotein (mg/dl), median ± IQR	52.9 ± 15.6
Any cardiovascular or antihypertensive medication treatment	1137 (40.9)
Mediterranean diet index ‡, median ± IQR	25 ± 4
Energy expenditure in leasure time (MET-min/week)*, median ± IQR	1515 ±1937
People with low education in the census tract (%), median ± IQR	11 ±12.2
Living at the same address for 10 years before IMT measurement	2252 (81)

* MET: Metabolic equivalent ‡ Adherence to Mediterranean diet was defined as quartiles of the index

Table 2. Estimated percent difference in intima media thickness (IMT) associated with a 10-yr average exposure contrast between the 5th and 95th percentiles. Exposure contrast indicated in parenthesis.

Exposure		IMTcca			IMT6seg	
	N	% change	(95% CI)	N	% change	(95% CI)
NO$_2$ (25 µg/m^3)						
crude	2780	3.67	(1.37, 5.98)	2188	4.98	(2.65, 7.31)
adjusted for sex	2780	3.67	(1.38, 5.96)	2188	4.88	(2.58, 7.18)
adjusted for age & sex	2780	0.04	(-1.83, 1.92)	2188	0.84	(-1.02, 2.71)
Model 1	2738	0.35	(-1.63, 2.32)	2155	0.71	(-1.25, 2.67)
Model 2 (possible intermediates)	2632	0.56	(-1.47, 2.59)	2074	0.52	(-1.52, 2.57)
Traffic load in a 100m buffer (7.200.000 veh m/day)						
crude	2780	5.25	(2.76, 7.74)	2188	6.38	(3.89, 8.88)
adjusted for sex	2780	5.21	(2.73, 7.68)	2188	6.31	(3.85, 8.78)
adjusted for age & sex	2780	1.39	(-0.64, 3.42)	2188	1.99	(-0.02, 4)
Model 1	2738	1.78	(-0.33, 3.89)	2155	2.08	(0, 4.17)
Model 2 (possible intermediates)	2609	1.91	(-0.24, 4.06)	2053	2.06	(-0.09, 4.21)
Traffic intensity in nearest street (15.000 veh/day)						
crude	2780	4.18	(2.01, 6.35)	2188	4.55	(2.43, 6.68)
adjusted for sex	2780	4.13	(1.98, 6.29)	2188	4.42	(2.32, 6.51)
adjusted for age & sex	2780	1.74	(-0.02, 3.5)	2188	1.75	(0.05, 3.44)
Model 1	2738	1.96	(0.14, 3.77)	2155	1.7	(-0.04, 3.44)
Model 2 (possible intermediates)	2632	2.32	(0.48, 4.17)	2074	1.8	(0.01, 3.59)

Model 1: sex, age, sex-age interaction, smoking status, education and marital status

Model 2: model 1 plus BMI, HDL, waist circumference, systolic and diastolic blood pressure, weekly energy expenditure in physical activity during leisure-time (tertiles), adherence to Mediterranean diet, plausibility of reported diet, medication treatment and percentage of people with low education at the census tract level. Models for traffic load were additionally adjusted for occupational status.

Table 3. Relative risk ratios for low or high ankle brachial index (ABI) (<0.9 or >1.3 compared

with ABI= 0.9 to 1.3, respectively) associated with 10-yr average exposure contrasts between the

5th and 95th percentiles. Exposure contrast indicated in parenthesis.

Exposure		ABI<0.9		ABI>1.3	
	N	RRR	(95% CI)	RRR	(95% CI)
NO$_2$ (25 μg/m^3)					
Model 1	2698	0.65	(0.28, 1.52)	1.91	(1.09, 3.33)
Model 2 (possible intermediates)	2600	0.72	(0.29, 1.75)	1.98	(1.09, 3.6)
Traffic load in a 100m buffer (7.200.000 veh m/day)					
Model 1	2698	0.92	(0.37, 2.3)	1.89	(1.1, 3.26)
Model 2 (possible intermediates)	2600	1.02	(0.4, 2.61)	1.89	(1.07, 3.34)
Traffic intensity in nearest street (15.000 veh/day)					
Model 1	2698	0.47	(0.16, 1.41)	1.6	(1.08, 2.38)
Model 2 (possible intermediates)	2600	0.48	(0.16, 1.46)	1.7	(1.13, 2.57)

Estimates adjusted by Model 1: sex, age, sex-age interaction, smoking status, education and marital

status; Model 2: sex, age, sex-age interaction, smoking status, education, marital status, BMI, HDL, waist

circumference, systolic and diastolic blood pressure, weekly energy expenditure in physical activity

during leisure-time (tertiles), adherence to Mediterranean diet, plausibility of reported diet, medication

treatment and percentage of people with low education at the census tract level. Models for traffic load

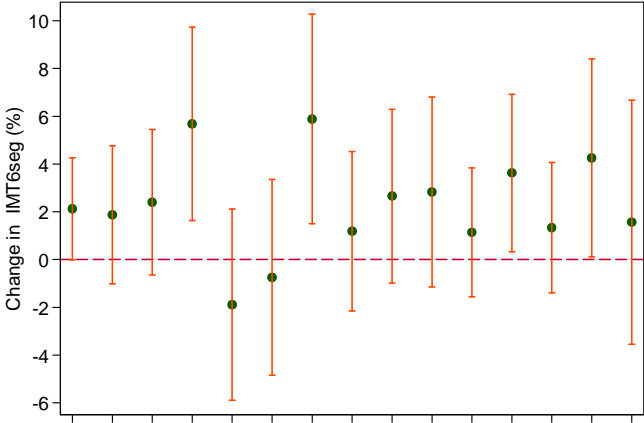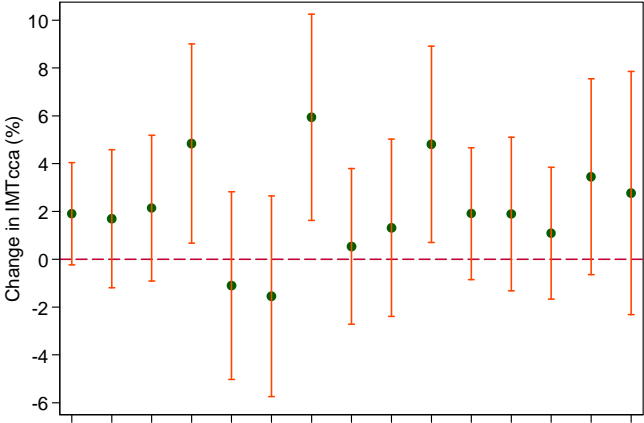were additionally adjusted for occupational status.

Figure Legend

Figure 1. Subgroup analyses: Estimates of percent differences in intima media thickness (IMT) associated with exposure contrasts between the 5th and 95[th]percentiles for 10-year weighted average values of NO_2 (25 ug/m3), traffic load within 100m (7,200,000 vehicle-meters/day), and traffic intensity on the nearest street (15,000 vehicles/day) according to sex, sex and age ≥60 years, education, medical treatment and smoking status.

Estimates adjusted by sex, age, sex-age interaction, smoking status, education, marital status, BMI, HDL, waist circumference, systolic and diastolic blood pressure, weekly energy expenditure in physical activity during leisure-time (tertiles), adherence to Mediterranean diet, plausibility of reported diet, medication treatment and percentage of people with low education at the census tract level. Models for traffic load were additionally adjusted for occupational status.

NO₂ (25 µg/m³)
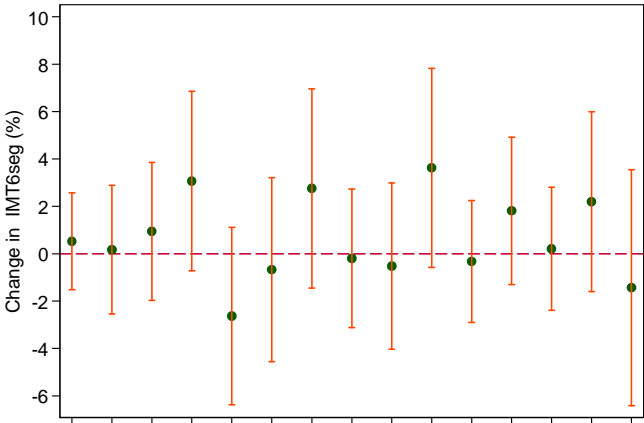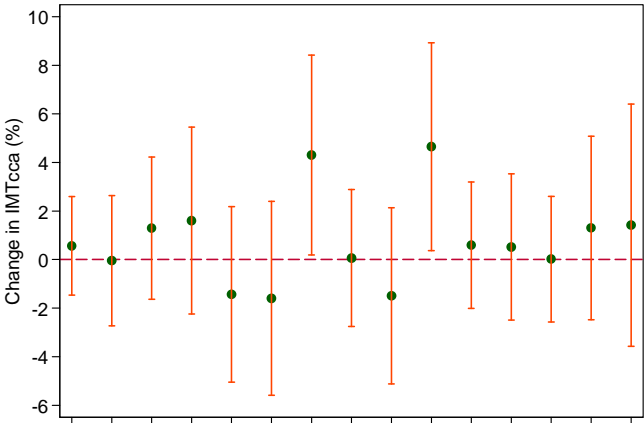
Traffic load in a 100m buffer (7.200.000 veh m/day)

Traffic intensity in nearest street (15.000 veh/day)